Food Hydrocolloids 63 (2017) 349–355

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Secondary structure and colloidal stability of beta-casein in microheterogeneous water-ethanol solutions

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ARTICLE INFO

Article history:
Received 11 June 2016
Received in revised form
2 August 2016
Accepted 13 September 2016
Available online 14 September 2016

Keywords:
Beta-casein
Secondary structure
Micellization
Water-ethanol solutions
Solvent microheterogeneity

ABSTRACT

Dynamic light-scattering (DLS), fluorescence spectroscopy (FS) and circular dichroism (CD) techniques were applied to study the influence of alcohol on beta-casein (b-CN) self-association and the secondary structure in a wide range of temperatures and ethanol concentrations. Temperature induced micellization and demicellization transitions of b-CN in water-ethanol solutions are revealed on the basis of the DLS data. The obtained results indicate that the association of b-CN at low and high alcohol concentrations proceeds through different mechanisms. It is suggested that the solvent microheterogeneity independently modulates both the secondary structure and the colloid properties of b-CN.

1. Introduction

Beta-casein (b-CN) is one of the members of casein family (Wong, Camirand, & Pavlath, 1996) and the most abundant milk protein. The amphiphilic character and high surface activity of b-CN are responsible for its good foaming and emulsifying properties (Dalgleish, 1997; Dalgleish, Spagnuolo, & Goff, 2004; Halling, 1981). b-CN molecules have a strong tendency to self-associate in aqueous environment forming globe-shaped surfactant-like micelles with hydrodynamic radius 7–14 nm (Dickinson, 1999; Faizullin, Konnova, Haertlé, & Zuev, 2013; Holt, 1998; Horne, 1998; Payens & van Markwijk, 1963; Portnaya et al., 2006; Rollema, 1992; Stroylova et al., 2013; Swaisgood, 1992). As was established by a variety of methods (Evans, Phillips, & Jones, 1979; Kajiwara et al., 1988; Niki, Takase, & Arima, 1977; Payens & Vreeman, 1982; Pearce, 1975; Portnaya et al., 2006; Schmidt & Payens, 1972; Swaisgood, 2003; Thurn, Burchard, & Niki, 1987) the critical micelle concentration of protein ranges from 0.3 to 0.7 mg/ml depending on temperature, pH and ionic strength of solution. The balance of two main driving forces – the attraction of hydrophobic domains and the electrostatic repulsion of the charged hydrophilic N-terminal regions forms the basis of the b-CN micellization process (De Kruif & Grinberg, 2002; Horne, 1998, 2002; Kumosinski, Brown, & Farell, 1993; Mikheeva, Grinberg, Grinberg, Khokhlov, & de Kruif, 2003; O'Connell, Grinberg, & de Kruif, 2003; Portnaya et al., 2008). The key point of this model is the ability of water molecules to form a continuous three-dimensional network of hydrogen bonds with enthalpy depending on temperature and configuration of bonds (Cinelli, Onori, & Santucci, 1997; Privalov, 1987; Ruckenstein & Shulgin, 2001). The presence of the third amphiphilic component in solution (ethanol in our case) which competes with water for the formation of hydrogen bonds affects spatial network of hydrogen bonds and weakens hydrophobic interactions. Consequently, all these effects can modify the colloidal state of b-CN.

Recently, b-CN has been classified as an "intrinsically unstructured protein" (IUP) (Tompa, 2002). Holt has argued (Holt & Sawyer, 1993) that caseins have the rheomorphic nature meaning that their secondary structure has rather large conformation space. Therefore, the secondary (probably, also tertiary) structure of b-CN

Abbreviations: b-CN, beta-casein; CD, circular dichroism; DLS, dynamic light-scattering; IUP, intrinsically unstructured proteins; PPII, polyproline II helix.

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http://dx.doi.org/10.1016/j.foodhyd.2016.09.011
0268-005X/